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Pulmonary Function and Symptom Responses after 6.6-Hour Exposure to 0.12 ppm Ozone with Moderate Exercise (Journal Version)

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16. ABSTRACT

Episodes occasionally occur when ambient ozone (O_3) levels remain at or near 0.12 ppm for more than 6 h. Small decrements in lung function have been reported following 2 hr exposures to 0.12 ppm O_3. For short exposures to higher O_3 concentrations, lung function decrements are a function of exposure duration. Thus, we investigated the hypothesis that prolonged exposure to 0.12 ppm O_3 would result in progressively larger changes in respiratory function and symptoms over time. Ten nonsmoking males (18-35 yr) were exposed once to clean air (CA) and once to 0.12 ppm O_3 for 6.75 h. Exposures consisted of six 50-min exercise periods, each followed by 10-min rest and measurement; a 45-min lunch period followed the third exercise period. Exercise ventilation averaged approximately 40 l/min. Forced expiratory and inspiratory spirometry and respiratory symptoms were measured prior to exposure and after each exercise. Airway reactivity to methacholine was determined after each exposure. FEV1.0 decreased progressively during the O_3 exposure and was decreased by 13.6% (588 ml) at the end of exposure; no changes were observed with CA. Decreases in FVC and FEF25-75 averaged 8.5% and 17.4%. On forced inspiratory tests, the FIVC and FIV05 were decreased 12.6 and 20.7% respectively. Increases in the symptom ratings of cough and pain on deep inspiration were observed with O_3 exposure but not with CA. Airway reactivity to methacholine was approximately doubled following O_3 expoure. We conclude that prolonged exposure to 0.12 ppm O_3 results in a marked increase in non-specific airway reactivity and progressive changes in respiratory function and symptoms.

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Pulmonary Function and Symptom Responses after 6.6-Hour Exposure to 0.12 ppm Ozone with Moderate Exercise

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Episodes occasionally occur when ambient ozone (O_3) levels remain at or near 0.12 ppm for more than 6 h. Small decrements in lung function have been reported following 2-h exposures to 0.12 ppm O_3. For short exposures to higher O_3 concentrations, lung function decrements are a function of exposure duration. Thus, we investigated the hypothesis that prolonged exposure to 0.12 ppm O_3 would result in progressively larger changes in respiratory function and symptoms over time. Ten nonsmoking males (18–33 yr) were exposed once to clean air and once to 0.12 ppm O_3 for 6.6 h. Exposures consisted of six 50-min exercise periods, each followed by 10-min rest and measurement; a 35-min lunch period followed the third exercise period. Exercise ventilation averaged approximately 40 L/min. Forced expiratory and inspiratory spirometry and respiratory symptoms were measured prior to exposure and after each exercise. Airway reactivity to methacholine was determined after each exposure. After correcting for the air exposures, FEV1.0 was found to decrease linearly during the O_3 exposure and was decreased by an average of 13.0 percent (591 mL) at the end of exposure. Decreases in FVC and FEF25–75% were also linear and averaged 8.3 and 17.4 percent, respectively, at the end of exposure. On forced inspiratory tests, the FIVC and FIV05 were decreased 12.6 and 20.7 percent, respectively. Increases in the symptom ratings of cough and pain on deep inspiration were observed with O_3 exposure but not with clean air. Airway reactivity to methacholine was approximately doubled following O_3 exposure. We conclude that prolonged exposure to 0.12 ppm O_3 results in progressive changes in respiratory function, a marked increase in nonspecific airway reactivity, and progressive changes in symptoms.

The magnitude of an individual's ozone-induced pulmonary function responses is a function of the inhaled exposure "dose" and his or her individual reactivity. It is well established that the principal components which contribute to the exposure dose are the ozone concentration, the volume of air breathed by the exposed individual (i.e. ventilation), and the duration of the exposure. The influence of ozone concentration and ventilation in modifying the exposure dose have been examined quite extensively in the past decade but the effect of exposure duration has not been subject to the same systematic investigation; studies of ozone exposure with durations in excess of two hours are rare. The scarcity of longer duration studies may be attributable to the possible misconception that most geographical areas with hourly average ozone concentrations in excess of 0.12 ppm have a pattern of ozone exposure similar to that which typically occurs in the South Coast Air Basin of Southern California, namely brief (1- to 3-h) peak exposures. However, other areas (such as parts of New York or New Jersey) exhibit ozone levels that, although not as high as in Southern California, are elevated over a broad time base, often in excess of 5 to 6 hours.[1]

A number of investigators[2-4] have reported decreases in respiratory function and increased respiratory symptoms after one or two hour exposures to 0.12 ppm ozone which included at least one hour of heavy exercise. In addition, field studies[5] of active children attending summer camps suggest a relationship between ambient ozone exposure and changes in lung function. We therefore hypothesized that young men performing the equivalent of a day of heavy work (or play) while exposed to 0.12 ppm ozone would experience respiratory discomfort and effects on lung function.

We were also interested in examining the time course of change in lung function, if indeed decrements were observed under these exposure conditions. Among the possible response patterns which might be observed were: a linear pattern of decline throughout the exposure; an initial decline followed by a response plateau; or minimal initial response (a "threshold") with an increased decrement toward the end of the exposure. Finally, a number of ozone exposure studies[6-8] have shown that airway reactivity to cholinergic agonists increases following exposure. It has also been reported that this increased reactivity is related to airway inflammation.[8] Since increased airway reactivity may be an indirect indicator of an airway inflammatory response, we also evaluated whether airway reactivity was influenced by these exposures.

Methods

The subjects for this study were 10 adult male nonsmokers (eight never-smokers, and two former "social" smokers—lifetime use <20 packs) recruited from the local population. The subjects were required to be at least moderately active (i.e., they participated in some form of regular exercise) so that they could sustain a prolonged period of moderate work. The screening procedures for each subject included a medical history, physical examination, routine hematologic and biochemical tests, and skin "scratch" tests for allergies to housedust and to the pollens of a set of 15 trees and grasses common to the area. None of the subjects had a history of

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allergic rhinitis, but four subjects had at least one positive skin-test response and one other subject was allergic to sulfa drugs. Subjects had no history or current evidence of cardiovascular or respiratory disease although one subject (subject 1) had an FEV1.0/FVC ratio (60 percent) which was below the normal range. Individual anthropomorphic and baseline lung function data are presented in Table I. The mean baseline FVC for these subjects fell between the mean values predicted from two reference standards[9,10] the mean baseline FEV1.0 was below the mean for each of the reference standards. The FEV1.0/FVC ratio averaged 78 percent whereas the expected FEV1.0/FVC ratio for these subjects was approximately 84 percent.

Prior to participation in the study the subjects were informed of the procedures and potential risks of the study and each signed a statement of informed consent; the protocol and consent form were approved by the University of North Carolina School of Medicine Committee on the Protection of the Rights of Human Subjects. Each subject participated in a training session during which he was instructed in methods of performing pulmonary function tests. At this time, the workload required to elicit a ventilation of 8 L/min per liter of FVC was determined for both treadmill and cycle ergometers. The exercise regimen of six 50-minute bouts of moderate exercise was intended to simulate work performed during a day of heavy to severe manual labor. A preliminary methacholine challenge was also performed after completion of the training session.

Experimental Design

Each subject underwent two exposure sessions, one to 0.12 ppm ozone and the other to clean air. These sessions were separated by a minimum of one week and exposure sequence was randomized. Subjects were exposed in pairs when convenient and the timing of entry into the chamber was staggered to allow for response measurements. Physiologic testing was performed just prior to exposure, during and after each of the six exercise periods, and at the end of the exposure.

Protocol

Upon arrival at the laboratory, a brief physical examination was performed on each subject. Baseline lung function measurements, which included forced expiratory tests, forced inspiratory tests, maximal voluntary ventilation, body plethysmography and symptom ratings, were performed prior to entering the chamber. Upon entering the chamber, which was already set for the appropriate conditions, the subject began working on a treadmill or a cycle ergometer at a previously determined setting. During the exposure, the subject performed a series of six exercise bouts alternating between the treadmill and cycle ergometer. Each exercise lasted 50 minutes and was followed by a 10-minute rest and measurement period. Following the third exercise, the subject was given an additional 35 minutes to eat lunch. During each exercise period we measured ventilation, tidal volume, breathing frequency, oxygen uptake, and heart rate after 40 minutes of exercise. Two forced expiratory maneuvers and a rating of four symptoms were obtained during the subsequent rest period. The only exception to this procedure was that airway resistance was also measured after the third exercise period. At the end of the 6.6-h exposure (i.e. following the sixth exercise period), the pre-exposure tests were repeated. Finally, a methacholine inhalation challenge was performed as described below; symptoms were rated again just prior to the challenge. Each subject was examined by a physician just prior to leaving the laboratory.

Measurement Methods

Methacholine Bronchial Challenge. All subjects were administered a series of aerosolized doses of methacholine chloride in normal saline; the methacholine concentrations were 0.0, 0.63, 1.25, 2.5, 5.0, 10.0, and 20.0 mg/mL. The procedures used in this study conformed to previously published guidelines.[11,12] The aerosol generation technique used was essentially the same as that previously reported for this laboratory[13] except that the output of the nebulizer was set at 3.0 mL/min. The aerosol was inhaled through a heated Fleisch pneumotach. A microprocessor controlled device was used which measured and regulated the tidal volume such that the subject inspired 5 breaths of aerosol at a tidal volume of approximately 25 percent of his vital capacity; end-inspiratory breath-holding time was 4 sec and the target inspiratory flow was 300 to 500 mL/sec. The subject first performed three Raw measurements prior to inhaling aerosol. He then began a series of aerosol inhalations, beginning with the lowest concentration, each of which were followed by two measurements of airway resistance, beginning 2 minutes after the start of aerosol inhalation. This sequence of aerosol inhalation and measurement was repeated at 6-minute intervals until the SRaw increased by more than 100 percent. The methacholine dose was determined as the product of inhaled volume of aerosol and methacholine concentration (L × mg/mL). The cumulative dose was expressed as the sum of the doses at all previous concentrations of inhaled aerosol. Initial determination of a potential subject's sensitivity to methacholine was made at least three days prior to any exposure. The subject's sensitivity to methacholine was taken as the cumulative dose of methacholine which produced a doubling of baseline (saline aerosol) specific airway resistance.

Pulmonary Function Measurements and Symptoms. Airway resistance (Raw) and thoracic gas volume (TGV) were measured in a body plethysmograph at a panting frequency of 1.5 Hz by a modified DuBois technique[15] described previously[13]; SRaw was calculated as the product of Raw and

Table I. Subject characteristics.

Number	Height (cm)	Weight (kg)	Age (yr)	FVC (mL)	FEV1.0 (mL)	FEV1/FVC (%)
1	190	79.5	29.8	6581	3971	60
2	191	102.0	27.7	5693	4598	81
3	181	75.0	32.9	5122	3891	74
4	183	65.5	23.2	5240	4283	82
5	187	68.8	23.0	5590	4712	83
6	175	61.4	27.1	5343	4432	83
7	178	75.0	23.5	4883	3720	76
8	176	75.2	20.6	5515	4076	73
9	179	78.9	26.5	5133	4338	85
10	178	66.7	18.0	5144	4362	85
Mean	181.8	74.8	25.3	5424	4238	78.2

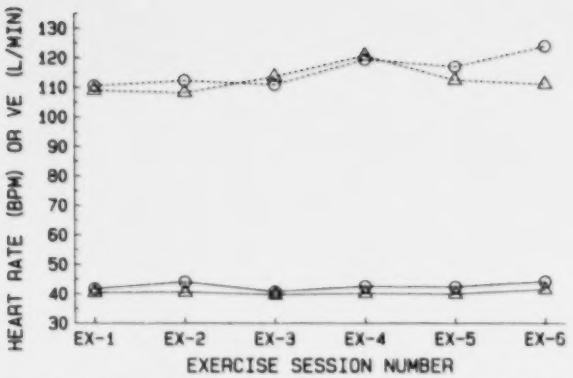

Figure 1. Mean ventilation and heart rate at the end of each of six 50-minute exercise periods. Heart rate is the dashed line and ventilation is the solid line; triangles represent air exposures and circles are ozone exposures. Standard errors were too small to include on this graph (S.E. for VE ranged from 1–2 L/min; S.E. for HR ranged from 3 to 5 BPM).

TGV. Spirometric measures were derived from a forced vital capacity (FVC) maneuver performed on a dry seal spirometer interfaced to a minicomputer; volumes were corrected to BTPS. For the present study, we report four expired spirometry measurements: FVC (forced vital capacity), FEV1.0 (exhaled volume during the first second of the FVC maneuver), FEV1.0/FVC, and FEF25–75% (mean expiratory flow during the middle half of FVC). Forced inspiratory maneuvers were used to derive forced inspiratory vital capacity (FIVC) and forced inspiratory volume at 0.5 seconds (FIV0.5). We also measured maximal voluntary ventilation (MVV). During each exercise session, the subject breathed through a pneumotachograph for three minutes in order to measure ventilation, respiratory frequency, tidal volume, and oxygen consumption, as described previously.[2]

Symptoms were evaluated prior to exposure, after each exercise period, and after leaving the chamber. Each symptom was evaluated on a five-point scale: 0 (none), 1 (just noticeable), 2 (mild discomfort), 3 (moderate discomfort), and 4 (severe discomfort or pain). Four symptoms were recorded: cough, discomfort when taking a deep breath (DDI), eye irritation, and pain or discomfort in the legs. Specific guidelines were provided for evaluating cough: 0 (none), 1 (cough during spirometry only), 2 (spontaneous single cough), 3 (spontaneous multiple cough), 4 (repeated spontaneous multiple cough).

Chambers

Exposures were conducted in a 4 × 6 × 3.2-m stainless steel chamber with continuous reconditioning and recirculation of the chamber air through high-efficiency particle filters. A detailed description of the chambers and their operating characteristics has been published.[14] Ozone was continuously monitored using a pair of Bendix chemiluminescent ozone analyzers which were calibrated daily by a Dasibi ultraviolet ozone photometer traceable to an NBS standard photometer. Ozone levels averaged 0.120 ± 0.002 (the minimum two minute average for all exposure sessions was 0.092 and the maximum was 0.141). NO, NO_2, and SO_x were also monitored; their concentrations were routinely less than 0.02 ppm, 0.005 ppm, and 0.005 ppm, respectively. The total particle mass with two persons in the chamber was routinely less than 3 $\mu g/m^3$ with 10^5 particles/m^3.

Data Reduction and Statistical Analysis

For spirometric data, the largest FVC and largest FEV1.0, regardless of the forced expiration from which they were derived, were used in the analysis. FEF25–75% was taken from the curve with the largest sum of FVC and FEV1.0. The largest FIVC and FIV0.5 were selected in a similar fashion. The average of two measurements of specific airway resistance was used in analysis (In some measurement sessions, more than two measurements were obtained; in these instances, the two most similar values for SRaw were averaged). The main hypotheses tested in this study were that there was no trend in the changes in FVC, FEV1.0, and FEF25–75% during the ozone exposure, after correcting for the changes during the air exposure, and that specific airway resistance was not altered by the ozone exposure. Multivariate analysis of variance methods appropriate for designs with repeated measurements were used to test these hypotheses. All other tests of hypotheses were of secondary importance and were done only to describe other potential ozone effects and clarify patterns in the data.

For the analysis of the methacholine challenge data, the "doubling dose" of methacholine was determined as follows. First, we plotted SRaw as a percentage of saline SRaw versus the log of the cumulative dose of methacholine at each methacholine dose level. The estimated cumulative dose (PD100) which would cause a 100 percent increase in specific airway resistance was linearly interpolated from this graph. The difference between the methacholine PD100 after air and ozone exposure was then analyzed by paired t-test.

We tested the null hypothesis that there was no association between the rating of the symptom variable DDI and the change in FVC. We computed the probability that the rank order of the decrease in FVC and the increase in DDI would occur by chance, using a permutation test. In addition, tests for associations between the three respiratory variables, FVC, FEV1.0, and PD100, and the two symptom variables, Cough and DDI, were done.

Results

Each subject completed two 6.6-h chamber exposures at the specified work output. The mean heart rate and mean ventilation for the work periods during the exposure are shown in Figure 1. The mean heart rates, which ranged from 108 to 124 beats/min correspond to a range of relative effort from 35 to 47 percent of predicted maximum oxygen uptake. Since there was a tendency for heart rate to increase modestly during this period of prolonged work, presumably due to fatigue and elevation of core temperature (not measured in the present study), the workload more nearly approximates the lower end of the observed range of relative effort (i.e. 35–40 percent of maximum oxygen uptake). The corresponding mean ventilations ranged from 39 to 44 L/min; the overall average was 40.4 L/min on the clean air day and 42.6 on the

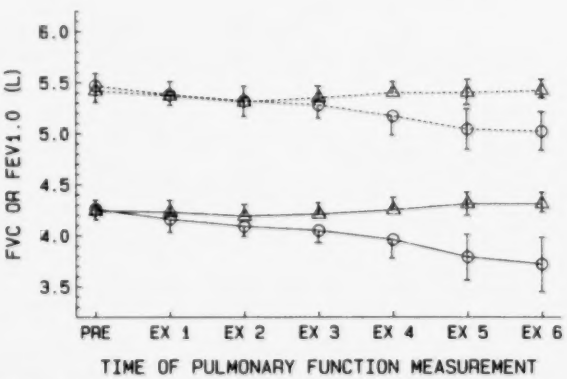

Figure 2. Mean ± S.E. of forced vital capacity and forced expired volume in one second after each of six exercise periods. Dashed lines are FVC and solid lines are FEV1.0; triangles represent air exposures and circles ozone exposures.

Table II. Lung function before and after 6.6-h exposures to clean air and 0.12 ppm ozone.[a]

	FVC	FEV1.0	FEF25–75%	FIVC	FIV0.5	MVV	SRaw
Clean air							
Preexposure	5424	4238	3.76	5284	3709	171	4.69
	±475	±317	±0.77	±458	±572	±25	±1.68
Postexposure	5417	4312	4.09	5368	3711	188	3.82
	±447	±342	±1.02	±520	±750	±28	±1.58
Pre-post Δ%[b]	−0.1	+1.9	+9.4	+1.6	+0.4	+10.5	−18.5
	±3.0	±6.0	±18.1	±3.3	±14.6	±12.6	±16.5
0.12 ppm ozone							
Preexposure	5472	4262	3.79	5386	3737	179	5.04
	±518	±293	±0.83	±486	±647	±22	±1.94
Postexposure	5017[c]	3721[c]	3.19[c]	4707[c]	2960[c]	175	5.73[d]
	±582	±787	±1.53	±775	±669	±33	±2.89
Pre-post Δ%	−8.3	−13.0	−17.4	−12.9	−20.5	−3.8	+11.7
	±6.2	±15.4	±29.7	±10.1	±13.4	±2.7	±30.5

[a] Units: FVC, FEV1.0, FIVC, FIV0.5 (mL); FEF25–75% (L/s); MVV (L/min); SRaw (cm $H_2O \cdot L^{-1} \cdot s \cdot L$).
[b] Mean ± S.D. of the percent change for each variable.
[c] $p < 0.01$.
[d] $p < 0.05$.

ozone day. Heart rate tended to rise slightly after the lunch break and during the last exercise period of the ozone exposure.

Main Analyses. The changes in spirometry, specifically FVC, FEV1.0, and FEF25–75%, which accompanied these exposures are shown in Table II for the pre- and post-exposure measurements. The mean FVC and FEV1.0 measurements at each of the seven measurement periods are shown in Figure 2. The decreases in these forced expiratory measures over the 6.6-h exposure averaged (i.e. average of the individual differences, not the mean difference) 8 percent, 13 percent, and 17 percent respectively.

The mean *differences* between the air and ozone days for FVC, FEV1.0, and FEF25–75% are presented, as a function of time, in Figure 3. The hypotheses of no linear, quadratic, cubic, etc. trend in these differences was tested. For all three variables, the hypothesis of no linear trend was rejected ($p = 0.007$ for FVC; $p = 0.004$ for FEV1.0; and $p = 0.021$ for FEF25–75%). The hypotheses of no quadratic, cubic, etc. trend could not be rejected for any variable. The conclusion is that all three variables decreased in an approximately linear manner throughout the 6.6 hours of ozone exposure.

Specific airway resistance increased during the ozone exposures and decreased during clean air exposures (Table II). Although the change in SRaw from baseline to postexposure was significantly greater for the ozone exposure compared to the air exposure, there was also a small but significant ($p < 0.05$) difference between the clean air preexposure baseline SRaw and the ozone preexposure baseline SRaw.

Secondary Analyses. The individual data for FEV1.0 are presented in Table III to illustrate the intersubject variability in response. We also tested for differences between the air and ozone exposures at each of the times at which spirometry was measured during the exposure. The differences between air and ozone exposure in spirometry measurements made at 4.6, 5.6, and 6.6 hours were significantly different for all three spirometry variables ($p < 0.05$).

Linear and three- and four-parameter logistic models were used to fit these data. Because the protocol was interrupted by a 35-minute rest period at midday, and because

Table III. Individual FEV1.0 measurements (mL) during 6.6-h exposure to clean air or 0.12 ppm ozone.

Subject	Preexposure	Post-exercise measurements Ex 1	Ex 2	Ex 3	Ex 4	Ex 5	Ex 6
			Clean air				
1	3971	4162	4222	4121	4160	4043	4165
2	4598	4449	4428	4544	4563	4618	4497
3	3891	3956	3869	3846	3912	3913	3933
4	4283	4430	4314	4380	4646	4555	4625
5	4712	4685	4557	4520	4602	4732	4555
6	4432	4508	4544	4416	4517	4766	4907
7	3720	3622	3762	3802	3854	3895	3826
8	4076	3930	3956	4059	4222	4193	4398
9	4338	4110	4008	4226	4074	4169	4016
10	4362	4458	4208	4145	3965	4171	4202
			0.12 ppm ozone				
1	4132	4124	4027	3678	3690	3417	3590
2	4458	4370	4090	4328	4283	4276	4322
3	3730	3645	3784	3829	3664	3573	3483
4	4365	4397	4338	4412	4483	4393	4516
5	4599	4599	4533	4715	4647	4707	4641
6	4611	4685	4685	4647	4647	4472	4342
7	3853	3636	3636	3540	2644	2547	2874
8	4355	4120	4039	3928	4042	3923	3935
9	4339	4155	4134	4092	3939	3578	3319
10	4175	3834	3743	3362	3561	3056	2189

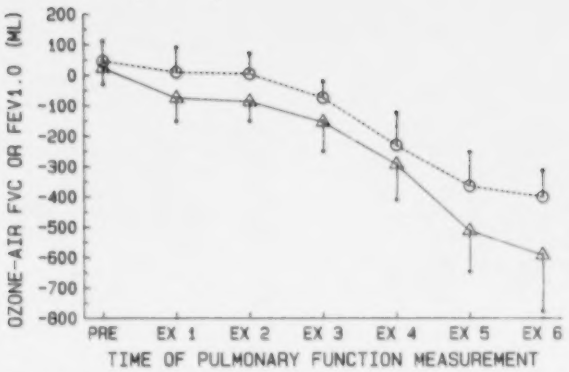

Figure 3. Ozone minus air exposure difference in FVC (circles) and FEV1.0 (triangles) measured after each of six exercise periods.

lung function did not change meaningfully during this time, we did not model this 35 minute period as part of the elapsed time. The best fitting line of decrease in FEV1.0 (Response) as a function of time (T) in hours was,

Response = $-51.8 + 99.6T$, $R^2 = 0.91$.

The best fitting three- and four-parameter logistic functions, respectively, were

Response = $751/(1 + e^{3.82-0.89T})$, $R^2 = 0.988$ and

Response = $47 + 602/(1 + e^{5.61-1.33T})$, $R^2 = 0.994$

There was a significant difference in maximal voluntary ventilation between the two post-exposure measurements although this was mainly due to an increase in MVV during the clean air exposures (Table II). Measures of forced inspiratory flow also demonstrated significant changes with ozone exposure (Table II). FIVC decreased 13 percent and FIV0.5 decreased an average of 20 percent ($p < 0.01$).

The respiratory pattern during exercise was examined across each of the six exercise sessions. There was no significant change in either tidal volume or respiratory frequency during the exposures. The overall average tidal volumes for air and ozone were 1596 and 1570 mL respectively; respiratory frequencies averaged 26 and 27 breaths/min, respectively.

Airway reactivity to methacholine was increased following exposure to ozone. A graph of percentage change in SRaw versus the log of the cumulative dose of methacholine (Figure 4) is shown for subject 8 for both air and ozone exposures to illustrate the ozone-induced shift in methacholine response. The individual values for the log-cumulative-dose which produced a doubling of SRaw (PD100) are shown in Table IV. The mean PD100 was 1.79 after air exposure and 1.47 after ozone exposure ($p < 0.01$). This difference indicates that twice as much methacholine was required, on average, to induce a doubling of airway resistance after air exposure compared to after ozone exposure (the antilog of the difference between the two PD100s is 2.09).

There was no effect of ozone exposure on symptoms of leg discomfort or eye irritation. However, coughing and symptoms of discomfort on taking a deep inspiration (DDI) increased during the ozone exposure. The distribution of the symptom ratings of DDI for both clean air and for ozone exposures is shown in Figure 5. In clean air there were only three reports (all by the same subject) of "just-noticeable" discomfort. During the ozone exposure, 50 percent of the subjects reported this symptom to be present after 3 hours of exposure. After the last exercise period (6-h), all subjects reported the DDI symptom and 50 percent of the subjects reported moderate or severe discomfort when taking a deep breath. To examine possible relationships between symptoms and function changes, the ratings of cough and DDI were tabulated with the changes in FVC and FEV1.0 at the end of exposure (Table V-A). The data are presented in descending order of the change in FVC and indicate that the largest FVC and FEV1.0 changes occurred in subjects with higher ratings of DDI. The probability that the observed order of FVC change and DDI ratings would occur by chance is $p < 0.01$. The association between changes in FEV1.0 and DDI was also significant although there was no association between cough and either FVC or FEV1.0. Similarly, the change in doubling dose of methacholine is tabulated in descending order with the symptom of cough (Table V-B). This table suggests a possible relationship between increased airway responsiveness and cough (Spearman $R = -0.63$; $p < 0.05$), but none with DDI.

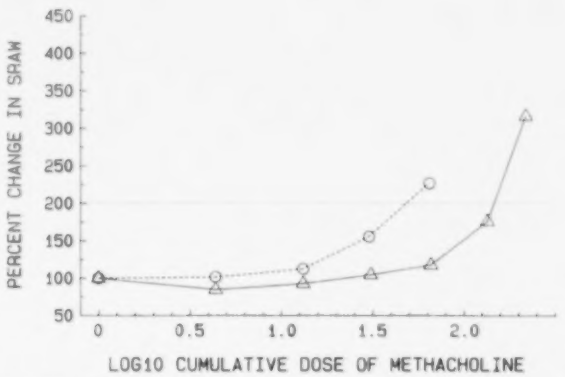

Figure 4. Representative dose-response curve of percent change in specific airway resistance (cm $H_2O \cdot L^{-1} \cdot s^{-1} \cdot L$) versus the log10 of the cumulative dose of inhaled methacholine aerosol following 6.6-h exposure to air (triangles, solid line) and 0.12 ppm-ozone (circles, dashed line). Dotted line at 200 percent indicates doubling of baseline SRaw; from the intersection of the dotted line and the respective curves perpendicular projections to the horizontal axis were used to determine the PD100, or methacholine dose which provoked a 100 percent increase in SRaw.

Table IV. Methacholine dose[a] required to double specific airway resistance after air and after ozone exposure.

Subject	Clean air	Ozone	Ozone-air difference
1	1.57	1.44	−0.13
2	1.02	0.71	−0.31
3	2.28	1.37	−0.91
4	2.35	2.07	−0.28
5	1.55	1.69	+0.14
6	2.10	1.85	−0.25
7	1.76	1.31	−0.45
8	2.18	1.73	−0.45
9	2.13	1.49	−0.64
10	0.99	0.99	0.00
Mean	1.79	1.47	−0.33
S.D.	0.50	0.40	0.31
	$t = 3.39$	$p < 0.01$	

[a] Dose = Log_{10} of methacholine solution concentration (mg/mL) × inhaled volume (L).

Discussion

There are several recent studies which indicate that significant pulmonary function responses may be elicited at or near 0.12 ppm ozone. McDonnell *et al.*[2] demonstrated small decreases (−4.5 percent) in FEV1.0 in young men exposed to 0.12 ppm ozone for two hours while performing heavy exercise. Similar small changes in lung function test measure-

ments (−3.4 percent FEV1.0) were subsequently observed in children exposed to ozone under similar conditions.[3] More recently Gong *et al.*[4] have demonstrated significant lung function (FEV1.0 decreased 5.6 percent) effects of 0.12 ppm ozone in trained athletes performing very heavy exercise for one hour. Field studies of active children attending summer camp have indicated a possible relationship between decreases in performance on lung function tests and ambient ozone levels below 0.12 ppm.[5,16] Conversely, other studies have not shown significant effects of ozone at or near the 0.12 ppm level.[17,18] In a study[19] of adolescent asthmatics, small (−3.1 percent FEV1.0) nonsignificant changes in lung function were reported for resting exposure to 0.12 ppm.

The present study was designed to examine the effects of a "workday" of exposure to 0.12 ppm ozone on lung function and airway reactivity in young men. The level and overall duration of exercise performed in this study simulated the performance of heavy physical labor for a full workday. The results of the present study and of selected previous studies of exposure to ozone concentrations ≤0.21 ppm, all with exposure durations of 1–2 hours and incorporating approximately 1 hour of heavy work, are shown in Table VI. We used the product of ozone concentration × ventilation × duration of exercise to calculate the "effective dose," a crude index of exposure. Based on this exposure index, we expected to find larger decrements than those reported in these previous shorter duration studies. The "expected decrements" were calculated as the product of the observed FEV1.0 decrement and the ratio of effective dose between our study and each of the studies in Table VI. Our observations fell between the extremes of the studies with similar effective dose exposures.[4,24,25]

Table V. Symptoms of cough and discomfort on deep inspiration (DDI) and changes in FVC, FEV1.0, FEF25–75%, SRaw, and airway reactivity to methacholine after 0.12 ppm ozone exposure.

A. Percentage change in FVC, FEV1.0, FEF25–75% and ratings of cough and discomfort on deep inspiration.[a]

Subject	% ΔFVC	% ΔFEV1.0	% ΔFEF25–75%	DDI	Cough
7	−19.0	−25.4	−37.9	4	3
9	−17.0	−23.5	−33.6	3	2
10	−11.8	−47.6	−87.1	3	1
1	−10.0	−13.1	−10.9	3	1
8	−8.4	−9.6	−9.3	2	0
5	−6.3	+0.9	+17.2	2	0
6	−6.0	−5.8	−5.4	2	2
4	−2.3	+3.5	+11.4	2	2
3	−2.2	−6.6	−10.0	1	3
2	−0.3	−3.1	−8.8	2	2

[a] % ΔFVC, % ΔFEV1.0, and % ΔFEF25–75% are (post-pre)/pre × 100%. FVC, FEV1.0, and FEF25–75% are significantly associated with DDI (Spearman $R = -0.89, -0.74$, and 0.70 respectively).

B. Change in methacholine dose required to double SRaw (PD100), percentage change in SRaw, and ratings of cough and DDI.[b]

Subject	Δ Log-dose methacholine	Cough	DDI	% ΔSRaw
3	−0.91	3	1	−6.9
9	−0.64	2	3	+42.3
7	−0.45	3	4	+42.0
8	−0.45	0	2	+41.8
2	−0.31	2	2	+24.5
4	−0.28	2	3	+8.3
6	−0.25	2	2	−17.3
1	−0.13	1	3	+14.7
10	0.00	1	3	+20.1
5	+0.14	0	2	−52.8

[b] Δ Log-dose methacholine = (Air dose − Ozone dose). % ΔSRaw is (post-pre)/pre × 100%. PD100 was significantly associated with Cough (Spearman $R = -0.63$).

Table VI. Concentration·time·ventilation (*CTV*) product and % ΔFEV1.0 in subjects performing heavy exercise.[a]

Ozone conc.	*CTV* product	% ΔFEV1.0	Exercise mode[b]	Reference
0.12	490	−4.5	IE	McDonnell *et al.*, 1983
0.12	698	−5.6	CE	Gong *et al.*, 1986
0.16	547	−6.1	CE	Avol *et al.*, 1984
0.16	653	−2.3	IE	Linn *et al.*, 1986
0.18	698	−6.4	IE	McDonnell *et al.*, 1983
0.18	1116	−11.0	CE	Horstman *et al.*, 1986
0.20	1164	−21.6	CE	Gong *et al.*, 1986
0.20	762	−3.3	IE	Kulle *et al.*, 1985
0.21	1134	−14.8	CE	Folinsbee *et al.*, 1984
0.12	1476	−12.7[c]	QC	Present study

[a] For exposures ≤ 0.18 ppm O_3, $r = 0.81$ for *CTV* vs ΔFEV1.0; a ΔFEV1.0 of −14.1 would be predicted for the present study. %ΔFEV1.0 is (post-mean − pre-mean)/pre-mean × 100%.
[b] IE = intermittent exercise; CE = continuous exercise; QC = quasi continuous exercise; ozone concentration in ppm.
[c] This number does not agree precisely with the mean percent difference reported in Table II because it was calculated as the percent difference of the pre- and post-exposure means, in the same manner as for the other studies listed in this table.

In addition to decrements in measurements of expiratory flow and volume, we found decreased inspiratory flow in these subjects. The mechanism for this decrease in performance on the inspiratory flow volume curve is not established but may be the result of a voluntary (or involuntary) decrease in inspiratory effort associated with the discomfort of taking a deep breath.

Another objective of the present study was to describe the response to ozone exposure as a function of time. Because we were interested only in the effects of ozone and because exercise alone may have some effect on lung function, we subtracted the values on the air day from those on the ozone day. The main statistical analyses indicated that the decreases in FEV1.0 followed a linear trend. The data for FVC and FEV1.0 suggest somewhat smaller changes early in the exposure with a more rapid rate of change occurring between 3 and 5 hours followed by a diminished rate of change over the final hour of exposure. In order to model these responses, the rate of change in FVC or FEV1.0 was expressed as change in lung function test as a function of exposure time (i.e., Δ FVC/h of exposure). The mean FEV1.0 data were fit quite well by linear, three-parameter logistic, and four-parameter logistic models (R^2 was 0.91, 0.988, 0.994, respectively). Care should be taken in trying to use these statistical models as a basis for inferring mechanisms of response or for extrapolating beyond the observed range of responses.

A diminished rate of change of function at the end of exposure has also been observed in previous two hour exposure studies[20,21] at higher ozone concentrations. Gliner *et al.*[20] reported that 70 percent of the decrement in FVC, observed over a two hour exposure to 0.42 ppm, occurred during the first hour. Similarly, Horstman *et al.*[21] reported that at the highest concentrations (i.e., 0.40 ppm) tested in their study,[2] 78 percent of the response occurred in the first hour whereas only 1 percent of the response occurred in the first hour of exposure at the lowest concentration, 0.12 ppm.

Although the experimental design was vastly different from the present study, the pattern of response is similar to that observed after exposure to increasing concentrations of ozone delivered during a series of fixed duration exposures.[2] The changes in FVC and FEV1.0 as a function of the total ozone effective dose in that study were small at the lowest exposure dose tested. With increasing ozone concentrations, the magnitude of change in FEV1.0 increased considerably

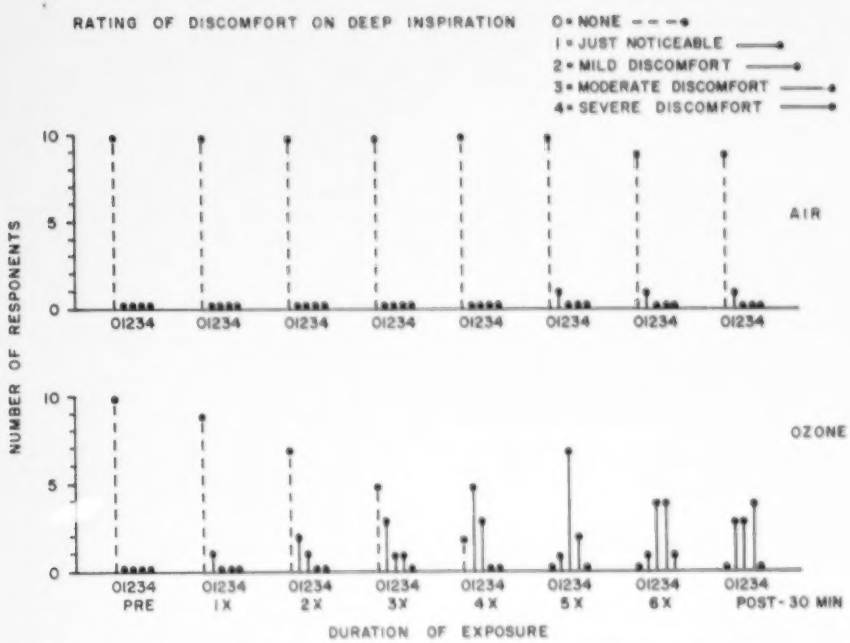

Figure 5. Rating of symptoms of discomfort on deep inspiration taken before exposure, after each of six exercise periods (1× through 6×), and prior to methacholine inhalation challenge, about 30 minutes after 6.6-h exposure to air or 0.12 ppm ozone. Numbers on the horizontal axis indicate symptom ratings: 0 = none (dashed line), 1 = just noticeable, 2 = mild discomfort, 3 = moderate discomfort, 4 = severe discomfort.

in the middle range of concentrations but at the highest concentrations tested, the magnitude of response did not show further increases. This group of observations,[2,20,21] including the present study, suggest that at low concentrations or exposure durations, pulmonary function responses are disproportionately small compared to longer duration or higher concentration exposures. In addition, there appears to be a limit to the size of the pulmonary function decrement which occurs following ozone exposure. However, there is a wide distribution of response to ozone,[2,4,20,27] which is exemplified in this study by the pronounced response of subject 10.

We also wanted to determine whether airway reactivity to methacholine was altered by prolonged low-level ozone exposure. Previous studies of airway responsiveness (to a single dose of histamine) after ozone exposure[6-8] have found airway hyperresponsiveness only after exposure to higher ozone concentrations (0.40–0.60 ppm). The mean change in the methacholine dose-response relationship in our study indicated that the dose required to cause a doubling of airway resistance was reduced by approximately 50 percent (i.e., one methacholine concentration level) following ozone exposure. Other investigators have observed a relationship between increased airway reactivity and increased numbers of neutrophils in bronchoalveolar lavage fluid following ozone exposure.[8,22,23] Airway hyperresponsiveness to methacholine may also be a marker of injury to airway epithelium.[24] The rating of the symptom cough tended to be elevated at the end of the ozone exposure in those subjects who experienced ozone-induced airway hyperresponsiveness to methacholine. There was, however, no relationship between airway hyperresponsiveness and other symptoms, notably discomfort on deep inspiration. On an individual subject basis, there was no apparent relationship between the change in methacholine reactivity and the changes in FVC and FEV1.0, suggesting that perhaps the changes in spirometry and changes in airway reactivity do not share a common mechanism.

In addition to the above functional changes, this study provided clear evidence, over the duration of the exposure, of increase in symptoms, such as cough or discomfort on taking a deep breath, associated with ozone exposure. The increase in discomfort from deeper breaths was clearly related in time to the decrease in FVC and FEV1.0 (Figure 5). Furthermore, of the four subjects with the largest decrements in both FVC and FEV1.0, each rated discomfort on deep inspiration at the level of 3 or 4 (i.e. moderate or severe discomfort). Of the remaining six subjects, only one rated discomfort above 2 (mild). This observation supports the notion that the decrease in FVC and FEV1.0 may be primarily related to a limitation of inspired volume either as a result of reflex or voluntary inhibition of inspiration.

In summary, significant lung function effects were observed after exposure to 0.12 pm ozone for a 6.6-h period which included 5 hours of moderate exercise. Decrements were observed in both inspiratory and expiratory spirometric measurements. Airway responsiveness to methacholine was increased and symptoms of cough and discomfort on deep inspiration were also significantly elevated. Although brief (one hour) exposure to 0.12 ppm results in little or no pulmonary function response, prolonged exposure (< 6 h) results in significant mean responses, with some subjects showing marked decrements in lung function measurements.

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The research described in this article has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency and approved for publication.

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